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Microfluidic System for Cell Fusion

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Abstract

We present a microfluidic chip for cell handling applications fabricated in polydimethylsiloxane (PDMS). The chip was designed to accumulate cells inside a reaction chamber where they can be manipulated under attendance of a liquid reagent (PEG – polyethylene glycol) for cell fusion. To enable the cell handling a fluid management system was developed which includes pneumatically actuated microfluidic valves integrated into the chip. Various valve geometries were simulated in ANSYS and characterized in a series of tests to identify a feasible valve concept. The main criterion of the valve development was a reliable closing characteristic. This characteristic was simulated in ANSYS and was verified experimentally. Both, the microfluidic layer and the pneumatic layer, were realized by means of soft-lithographic techniques and bonded using a plasma enhanced bonding process. First test series showed the eligibility of the applied valve concept and of the microfluidic chip for cell handling and for cell fusion.

Keywords: microfluidic chip; cell handling; cell fusion; valve; simulation; pneumatic actuation; polydimethylsiloxane;

1. Introduction

In modern biotechnology methods which are suitable for fusing different cell types to generate hybrid cells, which show hybrid characteristics of the two original cells, are of central interest. The hybridoma technology, for example, generates hybrid cell lines by fusing antibody-producing B-cells with a cancerous myeloma cell line. As a result of this fusion, the generated hybridomas produce monoclonal antibodies. Fusion is traditionally performed in a cell bulk of two mixed cell lines. User-controlled and observable cell fusion is not realizable with this method. In recent years, the use of microfluidic systems has become an eligible method for applications like biochemical assays, medical diagnostics, drug delivery, cell sorting and cell manipulation, as they enable transportation, isolation and manipulation of small amounts of liquids and cells. Polydimethylsiloxane (PDMS) is a well suited material for these applications, as microfluidic systems can be developed very cheaply and rapidly by methods of soft lithography [1, 2].

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2. Materials and methods

2.1. Microfluidic design and fabrication

Based on a previous study [3], the valve concept, which has been found most suitable for cell handling, is shown in Figure 1. The pneumatic chamber has a diameter of 1 mm and the height of 70 µm. The width and height of the fluidic channel is 200 µm and 40 µm, respectively. The thickness of the membrane between the fluidic layer and the pneumatic layer is varied between 4 µm and 30 µm. The rounded geometry of the fluidic channel is realized by heating the photoresist master to 140°C for 5 minutes, as described in [4]. The chip layout consists of three fluidic inlet channels (e.g. cell solution 1, cell solution 2, liquid reagent), one outlet channel and two backflush channels with one inlet and one outlet each (Fig. 2). The reaction chamber itself consists of a beaked structure including a centered passage with 5 µm in height (Fig. 3). This design is suitable for accumulating cells in the middle of the chamber and immobilizing them in close proximity. At the same time, it enables a gentle fluid stream, which is necessary for transporting more cell solution or another liquid medium into the chamber. More detailed information on the experimental setup is described in [3]. The masters of the microfluidic chip were fabricated by structuring AZ nLof / AZ 9260 photoresist (AZ Electronic Materials, Wiesbaden, Germany) on a silicon wafer. By casting liquid polymer onto the master wafer and curing it in a convection oven at 80°C for 20 minutes the channel network was transferred to the PDMS device. The PDMS (Sylgard 184, Dow Corning, Wiesbaden, Germany) is a two-component system, which was mixed in a ratio of 10:1 (base:curing agent). The casted PDMS layers were bonded in an O_2-plasma enhanced bonding process, leading to irreversible, tight bonding [2, 5, 6]. The device was finally bonded to another substrate to seal the remaining open channels (Fig. 1). After each bonding process, the device was baked at 80°C for 2 h. Further details concerning the fabrication process are described in [3].

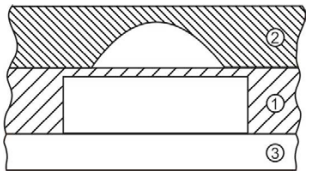

Fig. 1. Cross-section of valve area with round fluidic channel geometry. 1: pneumatic layer; 2: fluidic layer; 3: substrate

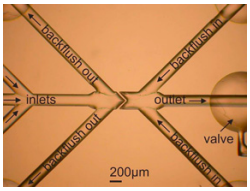

Fig. 2. Microfluidic network with inlet and outlet channels, backflush channels and pneumatic valves.

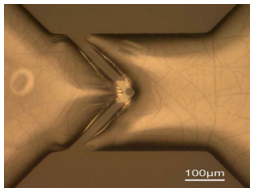

Fig. 3. Photoresist master of the reaction chamber. Cells can be accumulated in the central structure after molding.

2.2. Simulations

The valve geometries were simulated in ANSYS to investigate the pressure and geometry dependent closing characteristics of the valves. A static mechanical analysis of a symmetrical 0.7 mm x 0.7 mm x 0.4 mm section of the valves was performed, including 30000 nodes (Fig. 4). The Young's modulus of the PDMS was set to E = 2MPa and the Poisson's ratio to v = 0.499. The valves were simulated with pressure differences between the fluidic layer and the pneumatic layer in the range of of Δp = -0.5…-0.1 bar, whereas Δp is defined as $\Delta p = p_{fluidic} - p_{pneumatic}$.

2.3. Cell lines

In order to analyze the suitability of the microfluidic chip for cell handling, the human myeloid cell line U937 and the human lymphoid cell line L540 were chosen for the first series of tests. Passages through microfluidic structures may affect cell functions, e.g. by mechanical stress or adherence dependent activation. The viability of the cells after passage of the microfluidic was tested by viability and proliferation assays.

3. Results

3.1. Valve simulations

When the pneumatic layer is not pressurized, continuous flow is possible in the fluidic channel because the valve is intrinsically open. If pressure is applied to the pneumatic layer, the membrane bends upwards and blocks the channel so that the valve is in the closed state. The ANSYS simulations in Figure 4 show the valve actuation of a valve with a 30 µm thick membrane. When Δp = -0.5 bar, the membrane tightly adapts to the rounded fluidic channel, resulting in reliable sealing (Fig. 4 b). If the relative pressure difference is decreased to Δp = -0.4 bar or Δp = -0.3 bar, the valve switches from the closed state into the opened state and becomes more and more permeable (Fig. 4 c). Simulations of a 4 µm thick membrane show that an entire fluid blockage is already achieved for significant smaller pressure differences of Δp = -0.1 bar.

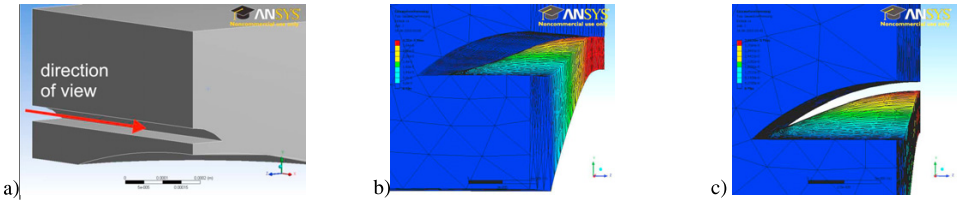

Fig. 4. ANSYS simulations of a 30 µm thick membrane. a) ANSYS model with illustrated direction of view for figures b and c. b) Δp = -0.5 bar, $p_{fluidic}$ = 0.5 bar, $p_{pneumatic}$ = 1.0 bar. c) Δp = -0.3 bar, $p_{fluidic}$ = 0.7 bar, $p_{pneumatic}$ = 1.0 bar.

3.2. Valve characterization

The valves are characterized by investigating the flow rate at certain pressure levels in the fluidic layer and in the pneumatic layer. Figures 5 and 6 show the closing characteristics of two valves which distinguish from each other in the thickness of their membranes. The valve with a 4 µm thick membrane shows an excellent flow blockage for a relative pressure of $\Delta p \leq$ -0.1 bar (Fig. 5). When the fluidic pressure is increased, while a constant pneumatic pressure is sustained, the flow rate also increases until a saturation curve is reached. This value only depends on the pressure applied to the fluidic layer. The transition between the closed state of the valve, where an entire flow blockage is achieved, and the opened state takes place within the range from Δp = -0.1 bar to Δp = 0.3 bar for all applied pneumatic pressures. Figure 6 shows the closing characteristic of the valve with a membrane of 30 µm thickness. Here, an entire fluid blockage can only be achieved for pressure differences of $\Delta p \leq$ -0.5 bar. The pressure range of the transition is likewise increased as it takes place in the range of Δp = -0.5 bar to Δp = 0.1 bar.

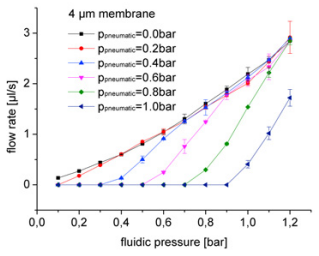

Fig. 5. Closing characteristics for a valve with a 4 µm membrane.

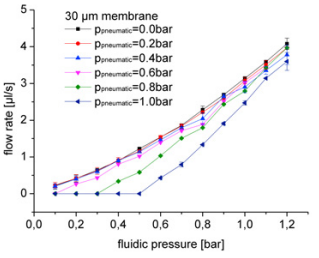

Fig. 6. Closing characteristics for a valve with a 30 µm membrane.

These experimental results verify and confirm the predicted valve properties based on the ANSYS simulations, as shown in Figure 4. The significant difference in the flow rate saturation values for a certain fluidic pressure is probably founded in a different flow resistance of the investigated systems (Fig. 5, 6).

3.3. Biological tolerance and cell fusion

The compatibility of the microfluidic chip for cell handling applications was tested by viability and proliferation assays, using the human cell lines U937 and L540. No significant difference in viability and proliferating activity was observed. Utilizing a user-controlled fluid management the cells can be immobilized inside the reaction chamber (Fig 7). Due to a subsequent flushing with PEG, successful fusion events were performed (Fig. 8).

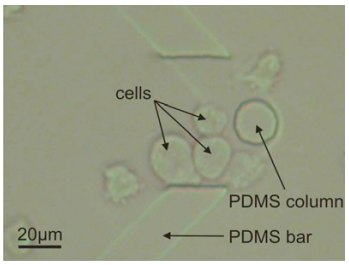

Fig. 7. Cell accumulation in the reaction chamber

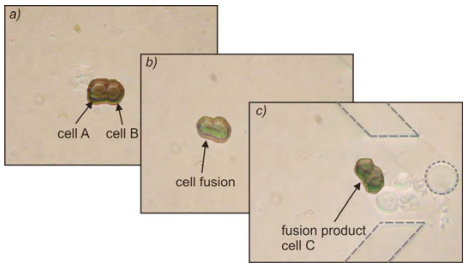

Fig. 8. Time series of a cell fusion event inside the reaction chamber. a) cell contact before fusion, b) fusion event, c) fused cells

4. Conclusions

In a series of tests we could verify the ANSYS simulation results of the valve actuation. The closing characteristic of the valves is adaptable by varying the thickness of the membrane. Furthermore, we showed that the microfluidic chip is suitable for user-controlled cell handling utilizing the human cell lines U937 and L540. We collected cells within the reaction chamber and performed successful cell fusion events utilizing PEG as initiator.

Acknowledgements

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